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(54) Title: USE OF PROTEINS AS AGENTS AGAINST AUTOIMMUNE DISEASES

(57) Abstract

The use of proteins extracted with perchloric acid from animal organs, for the preparation of medicaments active against autoimmune diseases, in particular with activity against atherosclerosis, arthritis, multiple sclerosis, diabetes.

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USE OF PROTEINS AS AGENTS AGAINST AUTOIMMUNE DISEASES

The present invention relates to the use of proteins extractable from animal organs, particularly from livers of mammals, for the preparation of medicaments active against autoimmune diseases, in particular activity against atherosclerosis, arthritis, multiple sclerosis, diabetes.

The administration of complete Freund's adjuvant has proved to be capable of inducing an experimental arthritis very similar to rheumatoid arthritis in rats. On the other hand, the administration of adjuvant to rabbits induces no arthritic pathology, but atherosclerosis. The studies carried out have evidenced that, in both lesions, immunoreactivity to an endogenous factor, which has been identified as the Heat Shock Protein 60 (HSP60), is present. Subsequent searches have confirmed these observations, proving that the administration of complete Freund's adjuvant can be replaced by the administration of HSP60, resulting in the same pathologies. Afterwards, pre-treatment of rat with adjuvant, HSP60 or fragments thereof has proved to prevent the onset of arthritis, with a still obscure mechanism, whereas the administration subsequent to the adjuvant worsens the progress of the disease.

More recently, pre-treatment with adjuvant has been found to also prevent other experimental pathologies which can be defined, generally speaking, as autoimmune disease, such as diabetes or experimental allergic enc phalomy litis (EAE). Finally, HSP60 has b en found to have structural analogies to a high number of

autoantigens, therefore it is assumed to be related to pathologies more widely than what up to now obs rv d.

WO 92/10197 disclosed protein fractions extractable with perchloric acid from organs of mammals, and their use as anticancer agents. Within these fractions, three main components could be identified, having molecular weights 50, 14 and 10 KDa on gel electrophoresis. The purified extract containing these three components will be referred to as UK 101 in the following. The sequence of the 14 KDa protein component, which is the main, if not the only, responsible for the described activities, is reported in the Table hereinbelow and in WO 96/02567, and it has turned out to be related to that described by other authors (Levy-Favatier, Eur. Biochem. 1903, 212 (3) 665-73) which have assumed that the novel identified sequences belong to the family of the proteins known as chaperonins, to which the HSPs themselves belong.

The proteins described in WO 92/10197 and those of WO 96/02567 (in the following referred to as UK 114) show anyhow properties never observed for chaperonins or analogous proteins. More specifically, it has been found that said proteins can be used in the prevention and in the treatment of autoimmune diseases, in particular atherosclerotic conditions, such as the atherosclerosis induced by organ transplants, arthritis, multiple sclerosis and diabetes.

The invention relates preferably to the use of the purified proteins UK 101 and UK 114 for the preparation of medicaments for the prevention and the treatment of autoimmune dis as s such as atherosclerosis following organ transplants, arthritis, multiple scl rosis,

diabetes.

Moreover the invention comprises the use of proteins showing a high homology degree to UK 114, of at least 80%, preferably of at least 90%.

ANTIATHEROSCLEROTIC ACTIVITY

It has been ascertained that nowadays the more frequent cause of failure of organ transplants in time is no more the rejection, but the formation of atherosclerotic plaques at the contact point between the vases of the transplanted organ and those of the host. This pathology is worsened by the usual immunosuppressors such as cyclosporin, whereas the use of AZT, which is however very toxic, appears to be useful.

The activity of the proteins UK 101 and UK 114 has been evidenced using both a conventional atherosclerosis model, which is that of the rabbit pre-treated with complete Freund's adjuvant, and a transplant atherosclerosis model. In the first case, the subcutaneous treatment with adjuvant induces within 21 days the formation of atherosclerotic plaques at the iliac bifurcation and at the aortic arch. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology in a high percent of cases compared with the treatment with the only adjuvant, which has lead to the development of the disease in all of the animals.

On the other hand, the experimental model of transplant atherosclerosis consists in the venous by-pass s at the level of arteri s in the rat. After a short time, the formation of atheroscl rotic plaqu s at

the level of the host vase, as it happens in the human pathology, has been observed. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology in a high percent of cases, compared with what observed in the animals non pre-treated before the transplant.

ANTIARTHRITIS ACTIVITY

This activity has been evidenced using a conventional arthritis model, which is the adjuvant-induced arthritis. In this model, Lewis rats are injected at the tail base with complete Freund's adjuvant: within 7 days, a pathology at the rear leg appears, characterized by swelling and joints alterations. The pathology reaches its peaks from the 14th to the 21st day, then decreasing until the leg returns to normal conditions. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology in a high percent of cases compared with treatment with the only adjuvant, which has lead to the development of the pathology in 100% of the animals. The treatment with UK 101 or UK 114 after the administration of adjuvant has worsened the progress of the pathology.

Therefore, it is considered that UK 101 and UK 114 are capable of modifying the progress of or of preventing pathological conditions such as arthritis and rheumatoid arthritis.

ACTIVITY AGAINST MULTIPLE SCLEROSIS

This has been evidenced using a conventional multiple sclerosis model: the experimental allergic encephalomyelitis (EAE). The pathology is induced

injecting subcutaneously Lewis rats with a Guinea-pig spinal cord homogenate together with complete Freund's adjuvant. The pathology appears as a progressive paralysis starting from the rear limbs, which begins at about the 12th day, reaches a maximum at about the 21st day and undergoes remission at about the 30th day from the administration of the immunogen. The pre-treatment (7 days before) with UK 101 or UK 114 has significantly prevented the development of the pathology in a high percent of cases and a less serious pathology has appeared, compared with treatment with the only marrow homogenate and adjuvant, which has lead to the development of the pathology in 100% of the animals.

Therefore UK 101 and UK 114 are believed to be able of changing the progress of or preventing pathological conditions such as multiple sclerosis.

ANTIDIABETIC ACTIVITY

This has been evidenced using a conventional diabetes model, represented by the BB rat which spontaneously develops diabetes around the 45th day of life. The animals have been treated at the 30th day of life with UK 101 or UK 114 and the development of the pathology has been observed, compared with untreated control animals. The pre-treatment has been found to decrease the incidence and the severity of the pathology in the experimental model. Some patients affected with tumors at different sites and also suffering from diabetes have been treated with UK 101 in the course of a compassionate treatment with the substance. All of the patients treated, independently of the effect on the tumor pathology, have shown a remission of the diabetic

pathology going so far as to quit the insulin therapy.

Therefore UK 101 and UK 114 are believed to be capable of changing the course of diabetes or of preventing it.

The antidiabetic activity has in fact been confirmed, although up to now in a limited number of cases, also in vivo in patients suffering from diabetes.

The proteins of the invention can be administered using suitable formulations, mainly injectable.

The pattern of the administration (doses, frequency of administration, etc.) will be determined according to the circumstances, depending on factors such as conditions of the patient, phase of the disease, etc., but usually a daily dosage ranging from 1 to 100 mg will be suitable.

TABLE

Met Ser Glu Asn Ser Glu Glu Pro Val Gly Glu Ala Lys Ala
1 5 10

Pro Ala Ala Ile Gly Pro Tyr Ser Gln Ala Val Leu Val Asp
15 20 25

Arg Thr Ile Tyr Ile Ser Gly Gln Leu Gly Met Asp Pro Ala
30 35 40

Ser Gly Gln Leu Val Pro Gly Gly Val Val Glu Glu Ala Lys
45 50 55

Gln Ala Leu Thr Asn Ile Gly Glu Ile Leu Lys Ala Ala Gly
60 65 70

Cys Asp Phe Thr Asn Val Val Lys Ala Thr Val Leu Leu Ala
75 80

Asp Ile Asn Asp Phe Ser Ala Val Asn Asp Val Tyr Lys Gln
85 90 95

Tyr Phe Gln Ser Ser Phe Pro Ala Arg Ala Ala Tyr Gln Val
100 105 110

Ala Ala Leu Pro Lys Gly Gly Arg Val Glu Ile Glu Ala Ile
115 120 125

Ala Val Gln Gly Pro Leu Thr Thr Ala S r Val
130 135

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: zetesis s.p.a.
(B) STREET: Galleria del Corso 2
(C) CITY: Milano
(E) COUNTRY: Italy
(F) POSTAL CODE (ZIP): 20122

(ii) TITLE OF INVENTION: Use of proteins as agents against autoimmune diseases

(iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 137 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Met Ser Glu Asn Ser Glu Glu Pro Val Gly Glu Ala Lys Ala
1 5 10

Pro Ala Ala Ile Gly Pro Tyr Ser Gln Ala Val Leu Val Asp
15 20 25

Arg Thr Ile Tyr Ile Ser Gly Gln Leu Gly Met Asp Pro Ala
30 35 40

Ser Gly Gln Leu Val Pro Gly Gly Val Val Glu Glu Ala Lys
45 50 55

Gln Ala Leu Thr Asn Ile Gly Glu Ile Leu Lys Ala Ala Gly
60 65 70

Cys Asp Phe Thr Asn Val Val Lys Ala Thr Val Leu Leu Ala
75 80

Asp Ile Asn Asp Phe Ser Ala Val Asn Asp Val Tyr Lys Gln
85 90 95

Tyr Phe Gln Ser Ser Phe Pro Ala Arg Ala Ala Tyr Gln Val
100 105 110

Ala Ala Leu Pro Lys Gly Gly Arg Val Glu Ile Glu Ala Ile
115 120 125

Ala Val Gln Gly Pro Leu Thr Thr Ala Ser Val
130 135

CLAIMS

1. The use of proteins extractable with perchloric acid from mammal liver, for the preparation of medicaments active against autoimmune diseases.

2. The use according to claim 1, wherein the protein has the following sequence:

Met Ser Glu Asn Ser Glu Glu Pro Val Gly Glu Ala Lys Ala
1 5 10

Pro Ala Ala Ile Gly Pro Tyr Ser Gln Ala Val Leu Val Asp
15 20 25

Arg Thr Ile Tyr Ile Ser Gly Gln Leu Gly Met Asp Pro Ala
30 35 40

Ser Gly Gln Leu Val Pro Gly Gly Val Val Glu Glu Ala Lys
45 50 55

Gln Ala Leu Thr Asn Ile Gly Glu Ile Leu Lys Ala Ala Gly
60 65 70

Cys Asp Phe Thr Asn Val Val Lys Ala Thr Val Leu Leu Ala
75 80

Asp Ile Asn Asp Phe Ser Ala Val Asn Asp Val Tyr Lys Gln
85 90 95

Tyr Phe Gln Ser Ser Phe Pro Ala Arg Ala Ala Tyr Gln Val
100 105 110

Ala Ala Leu Pro Lys Gly Gly Arg Val Glu Ile Glu Ala Ile
115 120 125

Ala Val Gln Gly Pro Leu Thr Thr Ala Ser Val
130 135

3. The use according to claim 1, wherein the proteins used have a homology of at least 80% to the protein of claim 2.

4. Pharmaceutical compositions containing as the active ingredient the proteins of claims 1-3 in admixture with suitable excipients.

5. The use according to claim 1, for the preparation of medicaments for th prevention and th treatm nt of atheroscl rosis following transplants.

6. The use according to claim 1, for the pr paration

of medicaments for the prevention and the treatment of arthritis.

7. The use according to claim 1, for the preparation of medicaments for the prevention and the treatment of multiple sclerosis.

8. The use according to claim 1, for the preparation of medicaments for the prevention and the treatment of diabetes.

INTERNATIONAL SEARCH REPORT

Inte. onal Application No
PCT/EP 97/05079

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6. A61K38/17 C07K14/47

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	WO 92 10197 A (BARTORELLI ALBERTO ;TURIANO ANGELA (IT)) 25 June 1992 see the whole document	4
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[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1998	18/02/1998

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INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/EP 97/05079

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropnate, of the relevant passages	Relevant to claim No.
X	LEVY-FAVATIER F ET AL: "CHARACTERIZATION, PURIFICATION AND CDNA CLONING OF A RAT PERCHLORIC-ACID-SOLUBLE 23-KDA PROTEIN RESENT ONLY IN LIVER AND KIDNEY" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 212, no. 3, March 1993, pages 665-673, XP000673794 see the whole document ---	4
X	WO 93 18146 A (INST NAT SANTE RECH MED) 16 September 1993 see the whole document see page 11, line 14 - line 23 ---	1,5-8
X	US 4 024 247 A (FORTINI JACK G ET AL) 17 May 1977 see the whole document -----	1,5-8

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Information on patent family members

Inte ional Application No
PCT/EP 97/05079

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